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1 **TITLE: Dung beetles reduce livestock gastrointestinal parasite availability**  
2 **on pasture**

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## Summary

1. Anthelmintics are commonly used to control gastrointestinal parasites of livestock. However, the residues of these compounds, particularly the macrocyclic lactones, are excreted largely unmetabolised in faeces where they may have toxic effects on dung colonising insects. Impoverishment of the coprophagous beetle community impairs the process of dung recycling and, as a result, may enhance the persistence of dung-dwelling helminth parasitic stages.
2. To test this possibility, a large-scale field trial was conducted in SW England. The availability of infective parasite helminth larvae (L<sub>3</sub>) was investigated on the herbage around 240 artificial 1 kg dung pats that had been constructed from the faeces of beef cattle with naturally acquired strongyle infections. Herbage up to 15 cm surrounding each pat was sampled at 2, 4, 6, 8 and 10 weeks after deposition. Pats were subject to enhanced, natural or no dung beetle colonisation and uncontrolled or enhanced rainfall.
3. Under uncontrolled rainfall conditions, 2 weeks after pat deposition, significantly more L<sub>3</sub> were recovered from around pats that were exposed to beetle colonization than from pats that were not colonised. However, by week 8, significantly fewer L<sub>3</sub> were recovered from around pats that were exposed to beetle colonization compared to uncolonized pats.
4. Under conditions of enhanced rainfall, pats yielded significantly more L<sub>3</sub> than under uncontrolled rainfall conditions, and there were no differences in recovery from herbage around pats with enhanced, natural, or no beetle colonization.
5. The data suggest that over the duration of a summer grazing season, temperate habitat dung colonizing insect communities, which include mainly small endocoprid dung beetles of the genus *Aphodius*, can reduce the development and survival of livestock gastrointestinal parasites on pastures, but that this can be overridden by the effect of high rainfall.
6. *Synthesis and applications.* The work demonstrates that conservation of dung beetle populations in temperate climates is important in livestock management, not only for their essential role in dung degradation and nutrient cycling, but because their activity can also reduce the survival and availability of gastrointestinal parasites on pastures.

**Keywords:** Agricultural ecosystems, anthelmintic, *Aphodius* spp., cattle dung, coprophagous beetle, endectocide, infective larvae, parasite ecology, strongyle, UK.

## Introduction

The management of gastrointestinal parasite infection is one of the most common and economically important challenges in livestock production (Charlier *et al.* 2009). Helminth eggs are shed from a parasitized host and then hatch and develop in the faeces until the infective third stage larvae (L<sub>3</sub>) migrate away from the dung to the surrounding herbage, where they are ingested by a ruminant host, thereby completing the cycle (Smith & Grenfell 1985).

The successful development, survival and migration of helminth larvae depends on environmental factors such as temperature and moisture (Stromberg 1997). For example optimal conditions for development of the cattle strongylids *Ostertagia ostertagi* (Stiles) and *Cooperia onchophora* (Railliet) are 23 °C and a 60-65 % faecal moisture content (Rossanigo & Gruner 1995). Any factors that make environmental conditions within a dung pat less suitable for larval survival are likely to reduce parasitic helminth populations and contribute to their management. Such effects have been attributed to the burrowing and burying activity of coprophagous beetles; beetle activity in surface faeces may speed up desiccation and aeration, making the dung unfavourable for L<sub>3</sub> development and migration (Houston, Craig & Fincher 1984). Indeed, the contribution of beetle activity to the ecosystem service of gastrointestinal parasite management has been estimated to save UK farmers £188 million per year in conventional cattle farming systems, at almost £20 per cow per year (Beynon, Wainwright & Christie 2015). However, while some work has supported the assumptions on which this calculation was based, the inherent complexity of interacting environmental and ecological factors make the role of beetles in helminth control difficult to demonstrate clearly.

In laboratory studies, the presence of endocoprid (dung dwelling) beetles was associated with a significantly greater recovery of *C. onchophora* and *O. ostertagia* L<sub>3</sub> from cattle faeces after 12 days, compared to beetle-free control dung (Chirico, Wikteliuss & Waller 2003). However, over the subsequent 12 days, this study found that L<sub>3</sub> recovery from the beetle-free dung increased significantly, whereas recovery from beetle colonised dung did not. An average reduction in faecal egg counts of 54% was recorded in 5-30 g sheep dung after 22-49 h of activity by 12-24 *Aphodius* spp. in the laboratory (Bergstrom, Maki &

Werner 1976). However, laboratory studies that sample L<sub>3</sub> directly from dung may be of limited value, since ingestion by the host and hence parasite transmission, is dependent on the availability of infective stage larvae on the herbage. Field studies, undertaken during an Australian summer, showed 60% reductions in L<sub>3</sub> recovery from herbage around strongyle-infected horse faeces; naturally colonised 1 kg faecal masses were compared to insect-free faeces and the difference was attributed to the activity of the paracoprid (dung burying) beetle, *Onthophagus gazella* (English 1979). Similar results were reported by Mfitilodze & Hutchinson (1988). Studies with paracoprid beetles are similarly difficult to interpret because strongyle larvae may protect themselves from desiccation by migrating into the soil, only moving to the herbage when conditions are adequately moist, which may be many months after faecal deposition (Bryan 1973, Krecek & Murrell 1988). Thus, the burial of contaminated dung by beetles may result in greater numbers of parasitic larvae being available in the long-term (Houston, Craig & Fincher 1984, Bryan & Kerr 1989). Any role of dung beetles in contributing to reduced parasite challenge depends therefore on the habitat, climate and the species of beetles available. Understanding these relationships is important in efforts to promote the sustainable management of grazed pastures. This is of particular current concern given that many of the anthelmintics administered to livestock, particularly the macrocyclic lactone compounds, are excreted almost unaltered in the faeces where they continue to exert an insecticidal effect, threatening dung beetle populations (Floate *et al.* 2005).

The aim of the present work, therefore, was to examine the effect of colonisation of cattle faeces by dung insects, particularly beetles, on the development of strongyle eggs within dung and the availability of infective larvae for migration onto pasture in a temperate habitat pasture system. In this environment, the majority of the beetles colonising pats are endocoprid dung-dwellers. The work compared the numbers of strongyle larvae in the herbage surrounding dung pats that were colonised naturally by dung insects, pats where dung insect colonisation was excluded and pats where the numbers of endocoprid beetles were artificially enhanced, under conditions of natural or enhanced precipitation.

## Materials and methods

### BEETLES

Dung beetles were collected in May and June 2015 from farmland in SW England, using dung-baited pitfall traps: for this 15 cm diameter buckets were buried flush with the ground, and 2 cm aperture wire mesh was placed on top. Artificial pats were placed on top of the wire mesh using a 20cm diameter pat former and fresh faeces from organic South Devon and Red Poll cattle. A 20 cm diameter rain shield was placed approximately 20 cm above each trap. Captured beetles were identified to genus in the field and *Aphodius* and *Onthophagus* spp. were collected, and stored in well-ventilated plastic containers with washed sand as a substratum and fresh organic cattle and horse faeces for food. Beetles were also collected by hand-searching naturally deposited pats from the same herd.

### FAECES

Faecal egg counts were performed on dung samples from a commercial herd of 60 organic cattle in SW England to confirm natural infection, using the mini-FLOTAC® method, which is accurate to 5 eggs per gram (Godber *et al.* 2015). None of the animals had been treated with anthelmintics for at least the previous 6 months. In early June 2015, 400 kg of fresh faeces was collected over a period of 2 days. At the end of each day the faeces was transported to the University of Bristol and stored in six 80 L plastic bins in a walk-in cold room (Cold Control Services, Ropley, UK) at 5°C. The following day all dung was combined and thoroughly mixed using a hand-held industrial plaster mixer (Silverline, Yeovil, UK). A 10g sample was taken from each bin of mixed dung for faecal egg counts.

Larval cultures were performed on the dung to identify the strongyle species present. Three 50 g samples were taken at random and placed in 14 cm diameter Petri-dishes in an incubator (Sanyo, London, UK) at 25 °C for 7 days. To prevent anaerobic conditions, which had been observed to prevent egg hatch in preliminary observations, 10 g vermiculite was mixed into each sample. Third stage larvae (L<sub>3</sub>) were harvested from the dung using a modified Baermann technique (Gruner 1986). Faecal samples were suspended in muslin over 250 mL inverse conical flasks filled with water, and left to stand for 24 h. The muslin and faeces were then removed and the supernatant was siphoned off leaving 20

mL of sediment. The sediment was agitated and transferred to 50 mL polypropylene centrifuge tubes (Fisher Scientific, Loughborough, UK) along with washings from the beaker to minimise the loss of larvae and placed in a centrifuge (IEC CL10, Thermo Scientific, Loughborough, UK) for 2 min at 1500 rpm. The supernatant was siphoned off to leave 5 mL of sediment, which was agitated and transferred to a 17 mL test tube (Beckman Coulter, High Wycombe, UK) along with washings from the centrifuge tube. The test tubes were centrifuged for 2 min at 1500 rpm and the supernatant was drawn off leaving 1 mL of sediment. This was agitated and transferred into plastic sample tubes (30 mL) (Fisher Scientific, Loughborough, UK) along with washings from the test tube and made up to 10 mL with water.

The suspension was placed on a glass slide with a drop of Lugol's iodine solution. The first 100 parasitic larvae observed were identified to genus and where possible species. Identification was carried out under a microscope at 200 x and 400 x total magnification by measuring total length, length of sheath tail extension, proportion of sheath tail extension comprising a filament, and using the morphological identification guide of van Wyk & Mayhew (2013). The shape of the head and presence of refractile bodies in the head, as found in *Cooperia* spp., were also used for identification.

#### EXPERIMENTAL FIELD TRIALS

Immediately after mixing, the dung was transported to a 180 x 20 m plot of grassland in SW England, which had not been grazed by cattle for at least the previous 5 years. Nematode extractions were performed on grass samples from across the site to confirm that no parasitic larvae were present. The faeces was used to form 260 artificial pats that were placed on the pasture in rows 3.5 m apart with 3.5 m between each pat. Pats were created using a 20 cm diameter plastic former that held 1 kg of faeces. A 10 g sample of faeces was taken after forming every 26<sup>th</sup> pat for faecal egg counting, to confirm that the distribution of strongyle eggs was consistent between pats.

Three factors were manipulated in the experimental design: dung colonisation (no colonisation, natural colonisation, enhanced beetle colonisation), rainfall (natural, enhanced), and dung disturbance (mechanically

disturbed, undisturbed). Twenty pats were allocated to each of the 12 unique treatment groups at random across the entire site. A further 20 pats (10 with natural colonisation and 10 with enhanced beetle colonisation) were created and then selected at random and removed after 10 days; they were then placed in emergence traps to allow insect colonisation to be quantified.

Five of the beetles that had been collected in the pit-fall traps were added to each of the pats in the enhanced beetle treatment group. Each pat received 1 *Aphodius fossor* (Linnaeus), 3 other *Aphodius* spp. from a pool of *A. fimetarius* (Linnaeus), *A. pedellus* (De Geer), *A. sphacelatus* (Panzer), *A. prodromus* (Brahm), *A. obliteratus* Panzer, and *A. contaminatus* (Herbst) and 1 *Onthophagus similis* (Scriba). This equated to adding approximately 67 mg dry beetle biomass per pat, an increase of 25% of the expected beetle biomass colonising pats under natural field conditions (Beynon *et al.* 2012).

In the mechanically disturbed treatment group a 2mm diameter rod was inserted into the pat to make 5 evenly spaced vertical 'tunnels' every day for the first 10 days. For the enhanced rainfall pats, water was applied to each pat three times per week for the duration of the entire experiment. A watering can and rose was held 1.5 m above the ground so that a 0.5 m diameter rain shadow was created over each 20 cm pat and surrounding 15 cm sampling area; 1 L of water was applied to each pat, over the 0.2 m<sup>2</sup> area; this equated to 5 mm of rainfall per application.

Immediately after formation of the 'no colonisation' pats, 30 cm diameter cages of 0.5 mm insect mesh were firmly pegged over them to prevent access by larger insects. In this treatment group, where pats were also to be disturbed mechanically and/or watered, the cages were removed, the treatment was administered and the cage was immediately replaced taking care that no beetles entered during this time. On day 10 all cages were removed to allow natural weathering of pats, as beetle colonisation was considered to be relatively unlikely after this time (Lee & Wall 2006). Pats to be placed in emergence traps were removed from the pasture on day 10 along with the underlying first 3 cm of soil. A weather station (OneCall, Leeds, UK) was placed at the field site to monitor temperature and precipitation throughout the trial.



## GRASS SAMPLING

On day 14 after the construction of the pats, all the herbage in the surrounding 15 cm area was cut to ground level taking care that no root mass, soil, or dung were included; the majority of infective strongyle larvae are expected to migrate up to 15 cm from the faeces (Williams & Bilkovich 1973). In the afternoon of the days prior to grass sampling, 2 L of water were applied to the pat and surrounding sampling area to encourage migration of available L<sub>3</sub> onto the herbage. Herbage was placed in grip seal polythene bags and returned to the laboratory. Sixty pats, selected at random, were cut each day over a period of 4 days so that all 240 pats were sampled. Nematode extractions were immediately set up on return to the laboratory, since the recovery of strongyle larvae from herbage samples has been shown to decline over time (Fine *et al.* 1993). This was repeated every 2 weeks for 10 weeks.

## NEMATODE EXTRACTION FROM HERBAGE

A modified version of the Baermann technique was used to extract the third stage parasitic nematode larvae from herbage (Gruner 1986). A 20 cm length of rubber tubing, with a 2 cm internal diameter (Fisher Scientific, Loughborough, UK), was attached to each of 60, 18 cm diameter plastic funnels and sealed with silicone sealant. Plastic specimen pots (2 cm diameter, 30 mL) (Fisher Scientific, Loughborough, UK) were pushed in to the other end of the rubber tubing. Grass samples were wrapped in 30 x 30 cm squares of muslin cloth to form a loose ball and secured with a rubber band. A wooden rod was passed through the band and the samples were suspended over the funnels. Funnels were filled with water that contained 2 ml of detergent per 12 L water, until 0.5 cm below the rim so that the entire muslin bag was submerged. Funnels were left to stand for 24 h; muslin bags were agitated by squeezing after 15 hours to encourage larvae to exit the bag. Herbage samples were then removed from the funnels, the tubes containing sedimented larvae were carefully collected from the ends of the rubber tubing, and placed in a refrigerator (Liebherr, Biggleswade, UK) at 5 °C for 1.5 h.

Water was siphoned off the top of each tube leaving 5 mL of sediment. The sediment was disturbed and transferred to a 17 mL test tube (Beckman

Coulter, High Wycombe, UK) along with washings from the original tube to minimise the loss of larvae. Test tubes were centrifuged for 2 mins at 1500 rpm. The supernatant was siphoned off leaving 1 mL of sediment, which was disturbed, transferred to 30 mL plastic specimen pots (Fisher Scientific, Loughborough, UK) along with washings from the test tube, and made up to 10 mL with water. The resulting larval suspensions were stored at 5°C until counted.

## NEMATODE COUNTING AND IDENTIFICATION

Aliquots of 1 mL were taken from each larval suspension and transferred into a Sedgewick Rafter nematode counting chamber with one drop of Lugol's iodine, under 40 × total magnification. Counting was repeated three times for each larval suspension and the average used for analysis. Parasitic larvae were differentiated from free-living larvae by the presence of a sheath, presence of a tail filament, staining of the intestinal cells; shape of the head, refractile bodies in the head and the absence of reproductive organs, using the morphological identification guide of van Wyk & Mayhew (2013).

## STATISTICAL METHODS

All statistical analysis was performed using RStudio (Version 0.99.489, 2009-2015) (R Core Team 2015). A generalized linear mixed model with a negative binomial error distribution was performed using the glmer.nb function of package 'lme4', with colonisation (none, natural, enhanced), dung disturbance (disturbed, undisturbed), and rainfall (natural, enhanced) as fixed effects, and L<sub>3</sub> count as the dependent variable. Experimental week (2, 4, 6, 8, 10) nested within individual pat was a random factor. Interactions between colonisation level, dung disturbance, rainfall, and experimental week were included. The model was simplified by stepwise removal of non-significant factors and the resulting minimal model contrasted with Akaike's Information Criterion (AIC) to the global model, as well as the conventional criterion of a statistically significant change in deviance between the models indicating a poorer fit, until the best fitting model was found (see Table S1 in Supporting Information).

T-tests were performed on the log<sub>10</sub>-transformed number (+1) of *Aphodius* spp. recovered from emergence traps containing pats that were exposed to either natural or enhanced beetle colonisation, and on faecal egg counts of dung samples after the dung was homogenised, and after it had been formed into pats. All means are presented ± their 95% confidence intervals, unless otherwise stated.

## Results

### FAECES

The dung collected from organic cattle and used to construct the artificial pats yielded an overall average of  $106 \pm 12$  strongyle eggs per gram (epg). Samples removed from each bin had an average of  $102 \pm 16$  epg and samples taken from every 26<sup>th</sup> pat in the field had an average of  $109 \pm 17$  epg; there was no significant difference between these groups ( $t_{14} = -0.6$ ,  $P = 0.57$ ). Hence, mixing of the 400 kg of collected dung appeared to have adequately homogenised the sample and it can be assumed that each of the 260 pats created contained a statistically similar number of strongyle eggs.

After laboratory incubation for 7 days at 25°C, larvae recovered from the cattle faeces used in this experiment consisted of 66% *Cooperia oncophora*, 29% *Ostertagia ostertagi* and 5% *Cooperia* spp.; the latter were not identified to species level. It must be noted that this is only an indication of the relative abundance of the species present, since optimal culture conditions for different species of gastrointestinal parasite larvae may differ (Whitlock, 1956; Dobson *et al.*, 1992).

### RAINFALL

Over the 10 week experiment the field site received 178.6 mm of rainfall, averaging 2.55 mm per day. The pats experiencing enhanced rainfall conditions received an extra 150 mm over the 10 weeks, giving them a combined average of 4.70 mm per day. Overall, the herbage surrounding pats in the enhanced rainfall group yielded significantly more L<sub>3</sub> than herbage surrounding pats in the natural rainfall group ( $z = 6.3_{1194}$ ,  $P < 0.001$ ) (Fig. 1).

### WEEK

The number of L<sub>3</sub> recovered from the herbage around pats increased significantly over the first 6 weeks of the experiment ( $z = 5.0_{1184}$ ,  $P < 0.001$ ) then remained relatively high until week 10 (Fig. 2).

### DUNG DISTURBANCE

There was no significant difference between the number of L<sub>3</sub> recovered from herbage around pats that were disturbed or undisturbed, and dung disturbance was not included in the final model.

#### DUNG COLONISATION

There was a significant interaction between colonisation treatment, rainfall and experimental week ( $z=-3.6_{1184}$ ,  $P<0.001$ ). At week 2, there was a significant interaction between colonisation treatment and rainfall ( $z=2.4_{232}$ ,  $P<0.05$ ). Under natural rainfall conditions, pats with no colonisation yielded significantly fewer L<sub>3</sub> than pats with natural colonisation or enhanced beetle numbers ( $t=-3.1_{2, 117}$ ,  $P<0.05$ ) (Fig. 3). Under enhanced rainfall conditions, there was no significant difference in the number of L<sub>3</sub> recovered from herbage around pats that had no or natural dung insect colonisation, or enhanced beetle numbers (Fig. 3). At weeks 4 and 6 there were no significant differences in the number of L<sub>3</sub> recovered from herbage around pats with no or natural insect colonisation, or enhanced beetle numbers (Fig. 3). At week 8 there was a significant interaction between colonisation level and rainfall ( $t= -2.6_{2, 234}$ ,  $P<0.05$ ). Under natural rainfall conditions, pats with no insect colonisation yielded significantly more L<sub>3</sub> than pats with natural colonisation or enhanced beetle numbers ( $t= 4.4_{2, 117}$ ,  $P<0.001$ ) (Fig. 3). Under enhanced rainfall conditions there was no significant difference in the number of L<sub>3</sub> recovered from herbage around pats that had no or natural colonisation or enhanced beetle numbers. At week 10, under natural rainfall conditions pats with no insect colonisation also yielded significantly more L<sub>3</sub> than pats with natural insect colonisation or enhanced beetle numbers ( $t= 2.1_{2, 234}$ ,  $P<0.05$ ) (Fig. 3). As at week 8, under enhanced rainfall conditions at week 10, there was no significant difference in the number of L<sub>3</sub> recovered from herbage around pats that had no or natural colonisation or enhanced beetle numbers.

#### BEETLE SPECIES RECOVERED FROM EMERGENCE TRAPS

Pats in this experiment were colonised by an average of 20 *Aphodius* spp. each, and had an average insect abundance of 199 including all Coleoptera and Diptera. A total of 407 *Aphodius* belonging to seven species were recovered from

the emergence traps and no *Onthophagus* spp. (Table. 1). There was no difference in the abundance of *Aphodius* spp. recovered from the traps covering the naturally colonised pats or the pats with enhanced beetle numbers ( $t = -0.33_{17}$ ,  $P = 0.75$ ). Pat colonisation approached a negative binomial distribution pattern (Fig. 5).

## Discussion

Most of the field studies on the impact of beetles on strongyle abundance have focused on dung-burying paracoprids (Bryan, 1973; Fincher, 1973; English, 1979; Bryan & Kerr, 1989; Nichols & Gómez, 2014). The present study examined a temperate system, where endocoprid dung-dwellers predominate (Jessop, 1986). In the present study, larvae had begun to migrate out of the pats in low numbers after 2 weeks in the field, increasing over weeks 4 to 6 and reaching a maximum by 8 weeks. Migration remained high for at least 10 weeks, as has been reported previously in the UK (Ogbourne 1972). The results show that the impact of dung colonising insects on the numbers of strongyle larvae available to emerge on to pastures is complex, changes over time and is strongly affected by environmental conditions. Under conditions of natural rainfall, in the first two weeks after dung pat construction, naturally colonised pats and those with additional beetles gave rise to a significantly greater number of *C. oncophora* and *O. ostertagi* L<sub>3</sub> on pasture herbage than uncolonised dung. Similarly, laboratory studies in Sweden by Chirico, Wikteliu & Waller (2003), using 500 g aliquots of cattle faeces naturally infected with trichostrongylids, mainly *C. oncophora* and *O. ostertagi*, to which forty endocoprid beetles (20 *Aphodius rufipes* and 20 *A. scybalarius*) had been added found that after 12 days a significantly greater number of L<sub>3</sub> were recovered from the dung with beetles than from beetle-free control dung. Strongyle egg hatch is dependent on oxygen availability (Albrecht 1909, Brown 1928, Nielsen *et al.* 2010), so it is possible that beetle activity initially provides aeration within freshly deposited faeces, preventing unfavourable anaerobic conditions from developing in the pat. This is especially likely in moist temperate climates. However, over time in the present study the initial pattern reversed; by week 8 significantly more L<sub>3</sub> were recovered from herbage around pats that were not colonised, compared to naturally colonised pats and those with additional beetles, under natural rainfall conditions. This pattern persisted until the end of the experiment - at least 10 weeks after pat deposition. A similar reverse was also seen by Chirico, Wikteliu & Waller (2003). In Australia strongyle larvae continued to migrate out of pats that were not colonised by dung beetles after 28 weeks but this was reduced to 24, 18 and

1.6 weeks for pats with minimal, moderate, and maximum natural beetle colonisation (Bryan and Kerr 1989).

The mechanism through which dung colonising insects might impact strongyle larvae remains unclear. Parasite egg hatch and larval development in the dung requires moisture, and migration of L<sub>3</sub> onto pasture herbage is dependent on adequate moisture to provide a film to facilitate movement (Stromberg 1997; Genever & Davies 2011). It is possible therefore that colonised pats undergo more rapid desiccation than uncolonised pats, leading to high mortality. This has been demonstrated for example in Australia; 1 kg pats of infected cattle faeces had a significantly lower water content after 7 days exposure to natural colonisation than faeces protected by insect mesh (Mfitilodze & Hutchinson, 1988). In temperate climates such as the UK, this process is likely to occur over several weeks rather than days. It was in an attempt to demonstrate this mechanism that some of the pats were disturbed by hand with a rod in an attempt to speed up desiccation in a similar manner to beetle 'tunnels'. No effect of this treatment was observed. However tunnels were only created for the first 10 days after pat construction, whereas colonised pats began to yield fewer L<sub>3</sub> than uncolonised pats only after eight weeks. So it is possible that the mechanical disturbance applied was insufficiently prolonged. Several authors have suggested that the ingestion of strongyle eggs and larvae by beetles may be responsible for some mortality (Miller, Chi-rodriquez & Nichols 1961; Bergstrom, Maki & Werner 1976; Grønvold *et al.* 1992). However, the maximum particle size ingested by species of large paracoprid, *Copris amyntor* and *Copris elphenor* was shown to be 20-45 µm and <5-50 µm by small endocoprids such as *Aphodius* (Holter, Scholtz & Wardhaugh 2002). Strongyle eggs are approximately 90 x 40 µm in size (Cuomo, Lawrence & White 2012). Hence, ingestion seems unlikely. Dung beetles probably avoid larger particles, which are mostly indigestible plant remains, and remove the smaller more nutritious particles which include bacteria and dead epithelial cells from the mammal gut (Holter 2000; Holter, Scholtz & Wardhaugh 2002).

The rate of development of gastrointestinal parasite larvae on pastures depends on temperature, optimal conditions being around 25°C for *O. ostertagi*,



but without moisture larvae do not develop (Stromberg 1997). Similarly the migration of L<sub>3</sub> away from faeces on to the herbage is dependent primarily on moisture, with temperature as the second most important factor (Krecek, Murrell & Douglass 1990; Stromberg, 1997). Splash dispersal by rain also has a role in the movement of infective larvae from faeces to the surrounding herbage (Grønvold 1984; Grønvold *et al.* 1992). L<sub>3</sub> release is therefore highly dependent on weather conditions. The field site received an average of 2.6 mm rain per day over the 10-week trial and the enhanced rainfall pats received 4.7 mm per day. Average rainfall (1981-2010) over the same June, July, August period for this area is approximately 1.9mm per day (Metoffice.gov.uk, 2016). The data presented here therefore suggest that under normal rainfall conditions, dung beetles will significantly reduce the availability of the infective stages of livestock gastrointestinal parasites on pastures, but that during periods of very high rainfall L<sub>3</sub> numbers are likely to be high regardless.

Under conditions of natural rainfall the differences between the colonised and uncolonised dung in the median numbers of L<sub>3</sub> counted in the herbage at 8-10 weeks were approximately 29%, supporting the figure of 31% used by Beynon, Wainwright & Christie (2015) in their calculation of the estimated reduction in strongyle numbers by dung beetles. However, effects of colonisation on L<sub>3</sub> availability was a dynamic process and the overall difference was lower in total; the difference in the total L<sub>3</sub> population between colonised and uncolonised dung over the entire 10 weeks was 19%. It should also be noted that the difference of 29% in strongyle numbers between colonised and uncolonised dung pats at 8-10 weeks was the effect of the entire dung colonising insect community; the inset mesh also excluded Diptera, the direct impacts of which on strongyle larvae are unknown. Hence estimates of the economic value of the decomposition ecosystem service provided by dung beetles in temperate climates need to reflect the subtlety of this effect.

A total of 407 dung beetles of seven species in the genus *Aphodius* were recovered from the 20 pats placed into emergence traps, and colonisation showed a highly aggregated distribution; significant levels of aggregation have been recorded previously in the majority of coleopteran and dipteran dung colonising taxa in SW England (Wall and Lee 2010). High replication is therefore

important in studies that consider the effects of natural dung colonisation on the development and transmission of strongyle larvae. In the weeks prior to the trial, approximately 500 beetles were collected in pitfall traps, which allowed 5 beetles to be added to each of the enhanced beetle colonisation pats (n=80) and the 10 enhanced colonisation pats to be placed in emergence traps, thereby increasing their beetle abundance by 25% on average. Had greater numbers of beetles been added to the pats, the impact on strongyle larval availability may have been more pronounced. Clearly given the almost bimodal distribution of beetle colonisation seen, beetle populations of up to 80 per pat would be within the normal range, but testing different beetle densities was outside the scope of this study. Nevertheless, the shape of the beetle colonisation distribution emphasises the considerable heterogeneity that there is likely to be in the impacts of beetles on strongyle availability within a single field.

The data presented here suggest that dung-colonising insect communities in temperate climates, which mainly include small endocoprid dung beetles in the genus *Aphodius*, will reduce the development and survival of livestock gastrointestinal parasites on pastures over the summer grazing season. The use of anthelmintics to control gastrointestinal parasites in livestock is commonplace, however many of these chemicals are excreted in the faeces largely unmetabolised, where they cause mortality in dung-colonising insects and their larvae (Steel & Wardhaugh, 2002). The conservation of dung beetles in temperate climates is therefore important in livestock management, not only for their role in dung degradation and nutrient cycling, but because they can contribute to the reduction in abundance of economically deleterious gastrointestinal parasites. Livestock management practices should focus on reducing reliance on anthelmintics to minimise damage to natural dung beetle populations.

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## **Data Accessibility**

Experimental data: Dryad Digital Repository (Sands & Wall 2016).

## **Supporting Information**

Additional supporting information may be found in the online version of this article.

**Table S1.** Model simplification using AIC (Akaike's Information Criterion) and Chi Squared P values for change in deviance, for identification of the best fitting negative binomial generalized linear mixed model for statistical analysis.

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702

703 **Table 1.** Insect abundance recovered from pats that had been removed from the field 10 days after deposition and placed in emergence

704 traps. There were ten replicates of each of naturally colonised and enhanced beetle pat.

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Treatment Repeat	Natural Colonisation										Enhanced Beetle Colonisation									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
<i>Aphodius fossor</i>	2	2	47	2	2	2	4	2	2	88	33	1	2	1	2	16	2	2	2	2
<i>Aphodius fimetarius</i>	2	3	1	1	1	2	1	3	1	4	2	6	2	2	2	2	2	2	2	2
<i>Aphodius pedellus</i>	2	2	1	2	2	1	2	2	2	2	1	4	2	2	2	2	2	2	2	2
<i>Aphodius ater</i>	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2
<i>Aphodius haemorrhoidalis</i>	90	14	23	2	2	2	2	2	2	1	2	2	2	8	2	11	2	4	2	2
<i>Aphodius erraticus</i>	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
<i>Aphodius depressus</i>	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2
<i>Aphodius</i> sp. (head only)	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2
<i>Sphaeridium scarabaeoides</i>	4	10	4	2	4	3	3	5	2	6	6	2	1	6	5	6	6	6	6	6
<i>Sphaeridium bipustulatum</i>	5	1	1	1	2	2	2	2	1	2	2	1	2	2	2	2	2	2	2	3
<i>Sphaeridium lunatum</i>	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	1	3	2	2	2
<i>Megasternum obscurum</i>	5	4	5	3	18	25	2	17	24	9	8	25	4	26	7	9	19	4	2	17
<i>Cercyon</i> spp.	4	4	3	2	8	14	1	6	2	1	4	3	5	2	1	7	6	7	7	7
<i>Paralister</i> spp.	2	2	2	2	2	3	3	2	2	2	2	2	2	2	3	2	2	4	2	2
Staphylinidae	88	31	54	55	86	55	25	61	22	51	40	47	87	23	47	30	33	76	29	26
Carabaeidae	2	4	2	2	5	2	2	1	3	2	3	2	2	1	2	2	2	2	2	2
Diptera	47	67	48	85	168	191	113	77	39	51	54	69	109	44	57	62	276	73	236	128
Ptilidae	10	10	14	2	2	3	6	18	6	2	7	34	12	4	15	4	15	21	21	21
Insect abundance	240	155	129	185	332	297	154	177	118	127	202	189	231	136	134	111	344	210	292	214
<i>Aphodius</i> spp. abundance	90	20	3	25	51	4	2	4	9	1	93	36	11	6	8	3	4	27	6	4
Average (±CI)																				

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## Figure legends

**Fig. 1.** The numbers of infective strongyle larvae (L<sub>3</sub>) recovered from the 15 cm area around pats that received natural rainfall or enhanced rainfall. The median L<sub>3</sub> is displayed within boxes representing the first and third quartiles; whiskers show 95% confidence intervals, outside of which fall the outlying points.

**Fig. 2.** The numbers of infective strongyle larvae (L<sub>3</sub>) recovered from the 15 cm area of herbage around pats every 2 weeks over the 10-week experimental period 11<sup>th</sup> June 2015 – 25<sup>th</sup> August 2015. Median L<sub>3</sub> is displayed within boxes representing the first and third quartiles; whiskers show 95% confidence intervals, outside of which fall the outlying points.

**Fig. 3.** The numbers of infective strongyle larvae (L<sub>3</sub>) recovered from the 15 cm area of herbage around pats under (a) natural and (b) enhanced rainfall conditions, over the 10-week period from 11<sup>th</sup> June 2015 – 25<sup>th</sup> August 2015. Pats were exposed to no insect colonisation (white box plots), natural insect colonisation (light grey), or natural insect colonisation plus enhanced beetle numbers (dark grey). Median L<sub>3</sub> is displayed within boxes representing the first and third quartiles; whiskers show 95% confidence intervals, outside of which fall the outlying points.

**Fig. 4** Frequency distribution of the number of dung beetles of the genus *Aphodius* recovered from 1 kg pats of naturally colonised cattle faeces.

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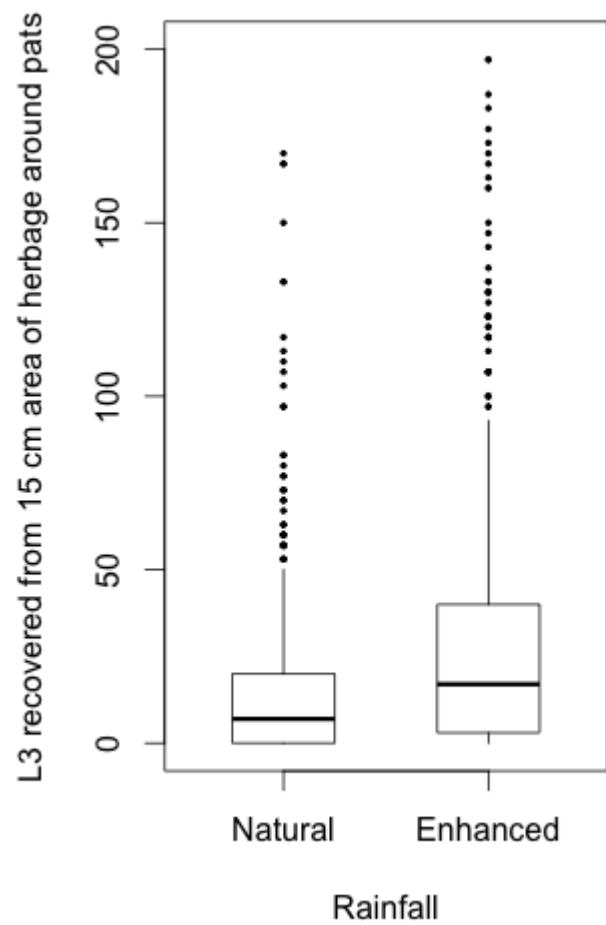


Fig. 1

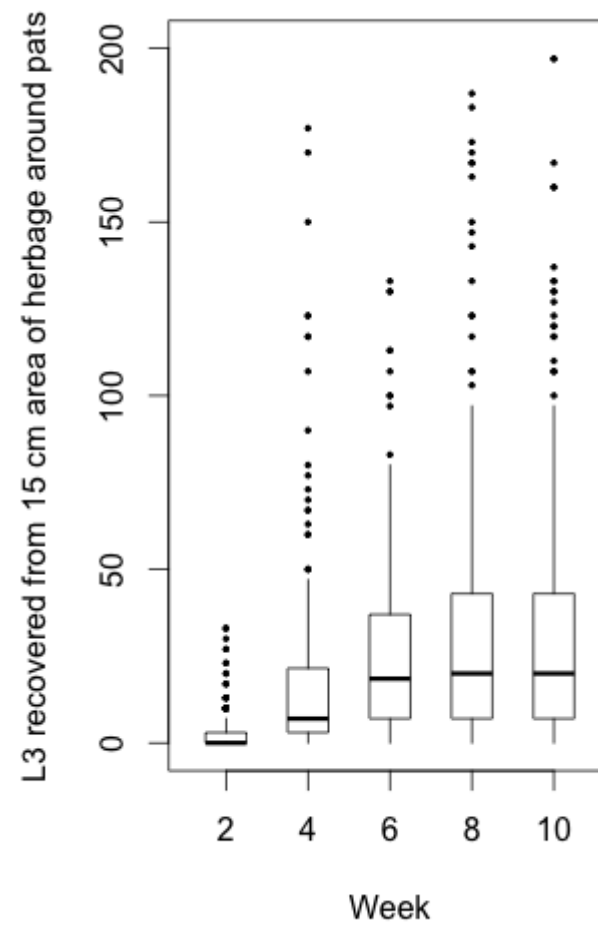
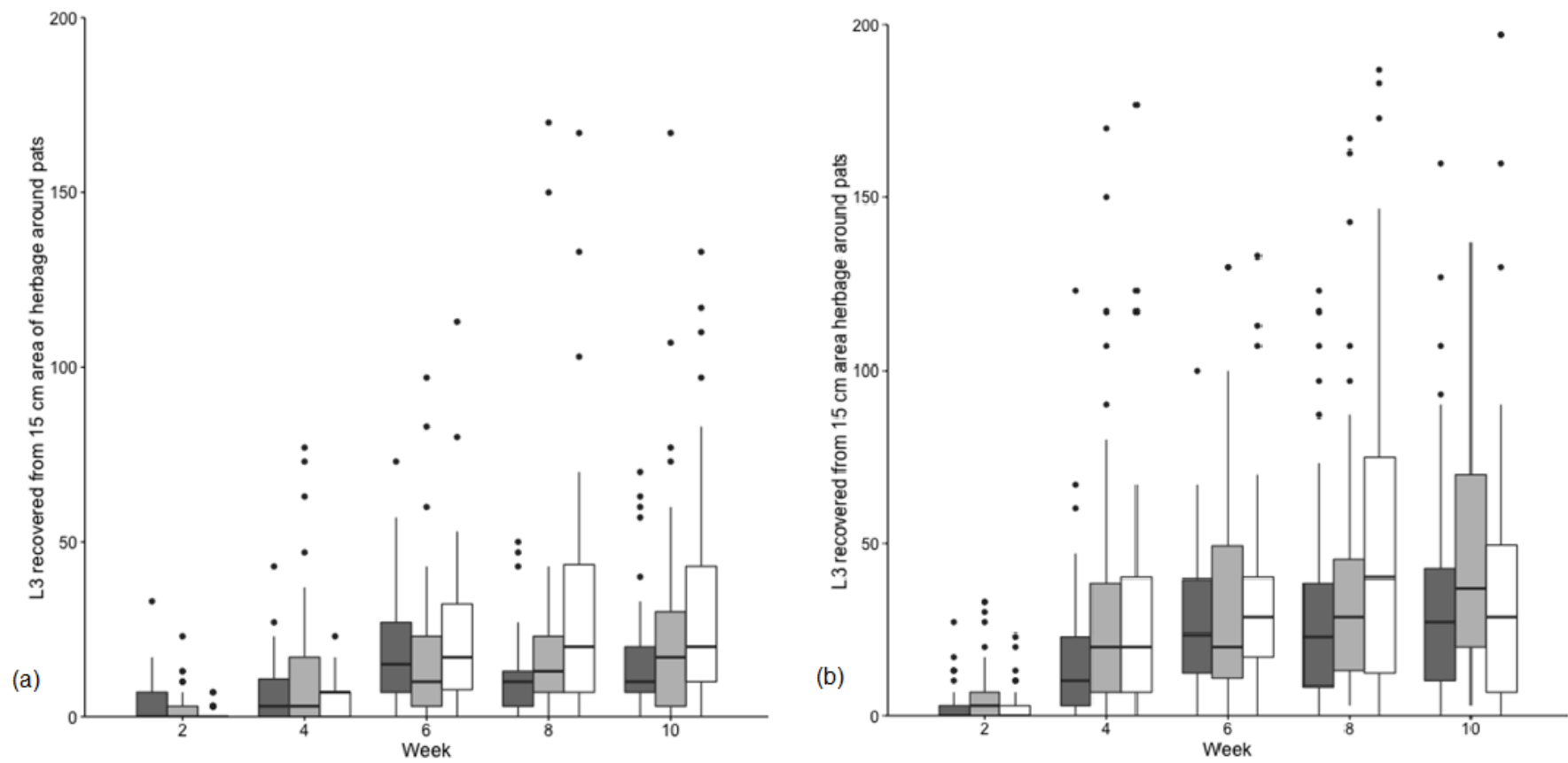


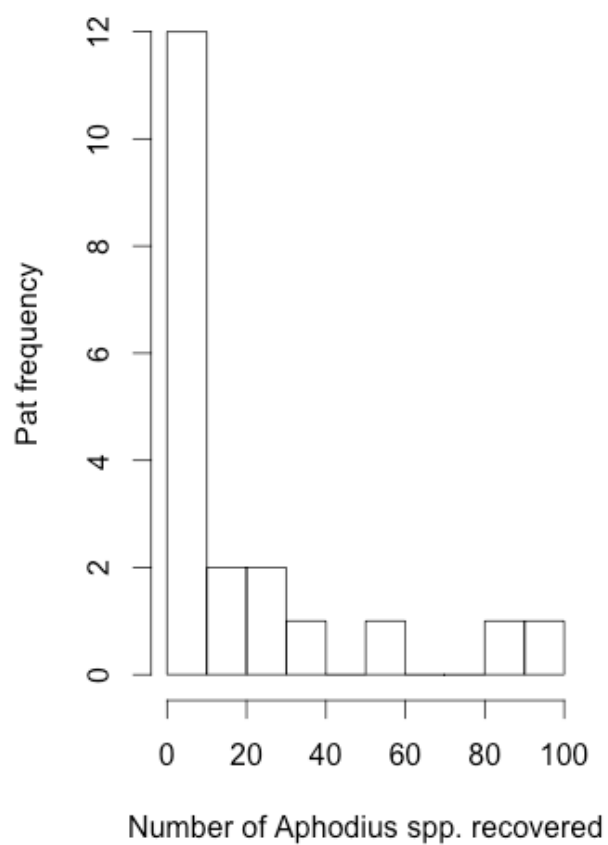
Fig. 2



**Fig. 3**

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744 **Fig. 4**

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